



Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection

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Diagnosis of HIV Infection in Infants (Updated August 11, 2011)

Panel's Recommendations:

- Virologic assays that directly detect HIV must be used to diagnose HIV infection in infants younger than 18 months **(AII)**. HIV antibody testing cannot establish HIV infection in this age group because maternal HIV antibodies may persist and interfere with the interpretation of a positive HIV antibody test.
- Virologic diagnostic testing is recommended in infants with known perinatal HIV exposure at ages 14–21 days, 1–2 months, and 4–6 months **(AII)**.
- Virologic diagnostic testing at birth should be considered for infants at high risk of HIV infection **(BIII)**.
- HIV DNA polymerase chain reaction (PCR) and HIV RNA assays are recommended as preferred virologic assays **(AII)**.
- Confirmation of HIV infection should be based on two positive virologic tests obtained from separate blood samples **(AI)**.
- Definitive exclusion of HIV infection **(in the absence of breastfeeding)** should be based on at least two negative virologic tests (one at >1 month and one at >4 months of age) **(AII)**.
- Some experts confirm the absence of HIV infection at 12–18 months of age in infants with prior negative virologic tests by performing an antibody test to document loss of maternal HIV antibodies **(BIII)**.
- In children ≥18 months of age, HIV antibody assays alone can be used for diagnosis **(AII)**.

Choice of Diagnostic Test

HIV infection can be definitively diagnosed through the use of virologic assays in most nonbreastfed HIV-infected infants by 1 month of age and in virtually all infected infants by 4 months of age. Tests for antibodies to HIV, including newer rapid tests, do not establish the presence of HIV infection in infants because of transplacental transfer of maternal antibodies; therefore a virologic test should be used¹. A positive virologic test (i.e., detection of HIV by DNA PCR or RNA assays) indicates likely HIV infection. The first test result should be confirmed as soon as possible by a repeat virologic test on a second specimen **because false-positive results can occur with both RNA and DNA assays**. HIV culture is not used for routine HIV diagnostic testing. The use of the currently approved HIV p24 antigen assay is not recommended for infant diagnosis in the United States because the sensitivity and specificity of the assay in the first months of life are less than that of other HIV virologic tests²⁻³.

HIV DNA PCR

HIV DNA PCR is a sensitive technique used to detect specific HIV viral DNA in peripheral blood mononuclear cells (PBMCs). The sensitivity of a single HIV DNA PCR test performed at <48 hours of age is less than 40% but increases to more than 90% by 2–4 weeks of age⁴⁻⁵.

HIV RNA Assays

HIV quantitative RNA assays detect extracellular viral RNA in the plasma and are as sensitive as HIV DNA PCR for early diagnosis of HIV infection in HIV-exposed infants. Studies have demonstrated sensitivities of 25%–40% during the first weeks of life, increasing to 90%–100% by 2–3 months of age⁴⁻⁸. Similarly, specificity is comparable between the two tests, although the detection of low levels of HIV RNA (<5,000 copies/mL) may not be reproducible and tests with low levels of HIV RNA should be re-

peated before they are interpreted as documenting HIV infection in an infant. An HIV RNA assay can be used as the confirmatory test for infants who have an initial positive HIV DNA PCR test. In addition to providing virologic confirmation of infection status, the expense of repeat HIV DNA PCR testing is spared and an HIV RNA measurement is available to assess baseline viral load. HIV RNA assays may be more sensitive than HIV DNA PCR for detecting HIV non-subtype B (see HIV subtype section below). **It is established that HIV DNA PCR remains positive even in individuals receiving therapeutic highly active antiretroviral therapy (HAART)⁹. However, whether the sensitivity of RNA assays might be affected by maternal antenatal therapy with combination antiretroviral (ARV) drugs and/or infant ARV prophylaxis is unknown.**

The HIV qualitative RNA assay (APTIMA HIV-1 RNA Qualitative Assay) is an alternative diagnostic test that can be used for infant testing¹⁰⁻¹³.

HIV Culture

HIV culture is not used for routine HIV diagnostic testing. It is generally not available outside of research laboratories. Although HIV culture has a sensitivity similar to that of HIV DNA PCR¹⁴, it is more complex and expensive to perform than DNA PCR or RNA assays and may require 2–4 weeks for definitive results.

Issues Related to Diagnosis of Non-Subtype B HIV Infection

Although HIV subtype B is the predominant viral subtype found in the United States, non-subtype B viruses predominate in some other parts of the world, such as subtype C in regions of Africa and India and subtype CRF01 in much of Southeast Asia¹⁵⁻¹⁷. Currently available HIV DNA PCR tests have decreased sensitivity for detection of non-subtype B HIV, and false-negative HIV DNA PCR test results have been reported in infants infected with non-subtype B HIV¹⁸⁻²¹. In an evaluation of perinatally infected infants diagnosed in New York State in 2001–2002, 16.7% of infants were infected with a non-subtype B strain of HIV, compared with 4.4% of infants diagnosed between 1998 and 1999²².

Some of the currently available HIV RNA assays have improved sensitivity for detection of non-subtype B HIV infection²³⁻²⁶, although even these assays may not detect or properly quantify some non-B subtypes, particularly the more uncommon group O HIV subtypes^{25, 27-28}. When non-subtype B perinatal exposure is suspected in infants with negative HIV DNA PCR, repeat testing using one of the newer RNA assays shown to be more sensitive in the detection of non-subtype B HIV (e.g., Amplicor HIV-1 Monitor 1.5 [Roche Molecular Systems, Pleasanton, CA], NucliSens HIV-1 QT [bioMérieux, Inc., Durham, NC], Versant Quantiplex HIV RNA 3.0 [branched DNA/bDNA] [Bayer Corporation, Tarrytown, NY], AmpliPrep/TaqMan HIV-1 Test [Roche Diagnostics, Indianapolis, IN], Real Time HIV-1 Assay [Abbott Molecular Incorporated, Des Plaines, IL], and the APTIMA HIV-1 RNA Qualitative Assay [Gen-Probe Incorporated San Diego, CA]) is recommended.

When evaluating an infant whose mother and/or father comes from an area endemic for non-subtype B HIV, such as Africa and Southeast Asia, clinicians should consider conducting initial testing using one of the assays more sensitive for non-subtype B virus (for example, one of the newer RNA assays mentioned above)^{25, 29}. When non-subtype B infection is suspected in a child with negative HIV DNA PCR and RNA assays, the clinician should consult with an expert in pediatric HIV infection. The child should undergo close clinical monitoring and definitive HIV serologic testing at age 18 months.

Timing of Diagnostic Testing in Infants with Known Perinatal HIV Exposure

Virologic diagnostic testing of the HIV-exposed infant should be performed at ages 14–21 days, 1–2

months, and 4–6 months. Virologic diagnostic testing at birth should be considered for infants at high risk of HIV infection (see below).

Two positive virologic tests obtained from separate blood samples provide confirmation of HIV infection on regardless of child's age. A positive HIV antibody test with confirmatory Western blot (or immunofluorescent antibody [IFA] assay) at age ≥ 18 months confirms HIV infection with the exception of rare late seroreverters (see HIV antibody section below)¹.

HIV infection can be *presumptively* excluded in nonbreastfed infants with two or more negative virologic tests, with one test obtained at ≥ 14 days of age and one obtained at ≥ 4 weeks of age; or one negative virologic test obtained at ≥ 8 weeks of age; or one negative HIV antibody test obtained at ≥ 6 months of age^{1,30}. *Pneumocystis jiroveci* pneumonia (PCP) prophylaxis is recommended for infants with indeterminate HIV infection status starting at 4–6 weeks of age until they are determined to be HIV uninfected or *presumptively* uninfected with HIV³¹. Thus, initiation of PCP prophylaxis can be avoided or, if prophylaxis was initiated, can be stopped, if the infant has negative virologic tests at 2 weeks of age and at ≥ 4 weeks of age, or if virologic testing is negative at ≥ 8 weeks of age.

Definitive exclusion of HIV infection in a nonbreastfed infant is based on two or more negative virologic tests, with one obtained at ≥ 1 month of age and one at ≥ 4 months of age, or two negative HIV antibody tests from separate specimens obtained at ≥ 6 months of age. For both *presumptive* and *definitive* exclusion of HIV infection, the child must have no other laboratory (e.g., no positive virologic test results or low CD4 count/percent) or clinical evidence of HIV infection and not be breastfeeding. Many experts confirm the absence of HIV infection in infants with negative virologic tests by performing an antibody test at 12–18 months of age to document seroreversion to HIV antibody negative status.

Virologic Testing at Birth (Optional)

Virologic testing at birth may be considered for newborns at high risk of HIV infection, such as infants born to HIV-infected mothers who did not receive prenatal care or prenatal ART or who had HIV viral loads $>1,000$ copies/mL close to the time of delivery. As many as 30%–40% of HIV-infected infants can be identified by 48 hours of age⁴. Blood samples from the umbilical cord should not be used for diagnostic evaluations due to the potential contamination with maternal blood. Working definitions have been proposed to differentiate acquisition of HIV infection during the intrauterine period from the intrapartum period. Infants who have a positive virologic test at or before age 48 hours are considered to have early (i.e., intrauterine) infection, whereas infants who have a negative virologic test during the first week of life and subsequent positive tests are considered to have late (i.e., intrapartum) infection³². Some researchers have proposed that infants with early infection may have more rapid disease progression than those with late infection and therefore should receive more aggressive therapy^{32–33}. However, data from prospective cohort studies have demonstrated that although early differences in HIV RNA levels were present between infants with a positive HIV culture within 48 hours of birth and those with a first positive culture after 7 days of age, these differences were no longer statistically significant after 2 months of age³⁴. HIV RNA levels after the first month of life were more predictive of rapid disease progression than the time at which HIV culture tests were positive³⁴.

Virologic Testing at Age 14–21 Days

The diagnostic sensitivity of virologic testing increases rapidly by age 2 weeks⁴, and early identification of infection would permit discontinuation of neonatal ARV prophylaxis and further evaluation for initiation of combination ART (see [When to Initiate Therapy in Antiretroviral-Naive HIV-Infected Infants Younger than 12 Months](#) and [Table 7](#)).

Virologic Testing at Age 1–2 Months

Infants with negative virologic tests before 1 month of age should be retested at 1–2 months of age. Most HIV-exposed neonates will receive 6 weeks of neonatal ARV prophylaxis. Although ARV agents could theoretically affect the predictive value of HIV virologic testing in neonates, the use of prenatal/intrapartum/neonatal zidovudine single-drug prophylaxis did not delay the detection of HIV by culture in infants in PACTG protocol 076 and has not decreased the sensitivity and predictive values of many virologic assays^{6-8, 30, 35-36}. The effect of prenatal and neonatal combination ARV regimens on the sensitivity of virologic tests for HIV-exposed infants needs to be examined. An infant with two negative virologic tests, one at ≥ 14 days and one at ≥ 1 month of age, can be viewed as *presumptively* uninfected and would not need PCP prophylaxis, assuming the child has no laboratory (e.g., no positive virologic test results or low CD4 count) or clinical evidence of HIV infection.

Virologic Testing at Age 4–6 Months

HIV-exposed children who have had repeatedly negative virologic assays at 14–21 days of age and at 1–2 months of age, **have no clinical evidence of HIV infection, and are not breastfed** should be retested at 4–6 months of age for *definitive* exclusion of HIV infection.

Antibody Testing at Age 6 Months or Older

Two or more negative HIV antibody tests performed at ≥ 6 months of age can also be used to *definitively* exclude HIV infection in HIV-exposed children with no clinical or virologic laboratory evidence of HIV infection.

Antibody Testing at Age 12–18 Months to Document Seroreversion

If there has not been previous confirmation of two negative antibody tests, many experts confirm the absence of HIV infection in infants with negative virologic tests by repeat serologic testing between 12 and 18 months of age to confirm that maternal HIV antibodies transferred to the infant *in utero* have disappeared. The proportion of infants who serorevert by 15–18 months of age is close to 100%, with as many as 95% seroreverting by 12 months of age. Factors that might influence the time to seroreversion include the staging of maternal disease and the sensitivity of the assay^{1, 37-40}.

Antibody Testing at Age 18 Months or Older

HIV infection can be diagnosed in children 18 months of age or older with a positive HIV antibody test and a confirmatory Western blot (or IFA assay).

On rare occasions, nonbreastfed HIV-exposed infants with no other route of HIV transmission (e.g., **receipt of contaminated blood products**, sexual abuse by HIV-infected person, or receipt of solid food pre-masticated by an HIV-infected caregiver) and no clinical or virologic laboratory evidence of HIV infection may have residual antibodies at 18 months of age. These infants should have repeat antibody testing because they may be late seroreverters, which can occur as late as 24 months of age⁴⁰. **In such cases, some experts would repeat virologic testing if the confirmatory HIV antibody test is positive at 18 months of age. This is due to reports, although rare, of late postnatal diagnoses despite negative virologic tests through 6 months of age as well as false-negative HIV DNA PCR assays in infants infected with non-subtype B HIV^{18-21, 41}.**

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